Odds Ratios and 95% Confidence Intervals for the Multinomial Logistic Regression of Symptom Latent Class Membership on Cytokines † Controlling for Age, Time Since Transplant, cGVHD Severity, Intensity of Current Immunosuppression and Absolute Lymphocyte Counts †

	Symptom Profile 1 (low on all symptoms) vs. Symptom Profile 2 (prominent oral/ GI symptoms) OR (95% CI)	Symptom Profile 1 (low on all symptoms) vs. Symptom Profile 3 (prominent eye, muscle/joint, fatigue and mood symptoms) OR (95% CI)	Symptom Profile 2 (prominent oral/ GI symptoms) vs. Symptom Profile 3 (prominent eye, muscle/joint, fatigue and mood symptoms) OR (95% CI)
IL-1 β	1.59 (0.30–8.45)	0.92 (0.32–2.60)	0.58 (0.10–3.32)
IL-6	0.27 (0.09–0.79)*	1.15 (0.52–2.52)	4.26 (1.32–13.82)*
IL-1 RA	0.69 (0.22–2.14)	0.55 (0.29–1.07)	0.81 (0.26–2.48)
sIL-6R	0.50 (0.12–2.13)	1.78 (0.54–5.90)	3.53 (0.83–15.13)
TNF-RII	0.29 (0.04–2.42)	0.28 (0.08–0.99)*	0.95 (0.12–7.59)
MCP-1	8.70 (1.46–51.66)*	0.40 (0.11–1.40)	0.05 (0.01–0.36)**
MIG	1.33 (0.73–2.44)	1.02 (0.63–1.65)	0.77 (0.42–1.39)
sBAFF	0.40 (0.13–1.30)	1.86 (0.88–3.93)	4.61 (1.39–15.31)**

† values were log normal transformed prior to analysis
**or *p < .01 or p < .05, respectively, multinomial logistic regression.

Higher IL-6 levels significantly differentiated participants in Group 2 from those in Groups 1 and 3. Group 3 had higher TNF-RII compared to Group 1. Those with higher sBAFF levels were significantly more likely to be in Group 2, while those with higher MCP-1 levels were significantly more likely to be in Group 3.

Conclusion: Allogeneic HSCT survivors with differing cGVHD symptom profiles were distinguished by significantly different levels of IL-6, TNF-RII, MCP-1 and sBAFF. These data validate this new symptom grouping system based on the Lee Chronic GVHD Symptom Scale as a measure of cGVHD disease burden. IL-6, TNF-RII, MCP-1 and sBAFF appear to be important biomarkers that reflect specific cGVHD manifestations and merit further study.

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DONOR DENDRITIC CELLS INITIATE COUNTER-REGULATORY IMMUNE RESPONSES AND GVL EFFECTS IN ALLLOGENEIC BMT

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Based on a clinical association of donor plasmacytoid dendritic cell (DC) content with leukemia relapses after allo BMT (Waller, Blood 2001), we previously reported that donor $CD11b^-$ DC enhanced IFN-γ synthesis and GvL activity of donor T-cells, while $CD11b^+$ DC resulted in increased IL-10 production and decreased GvL function by donor T-cells in allo BMT mouse models (Li, Blood 2007). In this study, we tested mechanisms whereby donor DC in the graft modulate donor T-cell activation in a MHC-mismatched (C57BL/6→B10.BR) allo BMT. Recipients received 11 Gy irradiation followed by tail vein injection of purified donor HSC, DC subsets and T-cells two days later. Allografts consisted of 5×10^4 FACS-purified donor BM $CD11b^-$ DC or $CD11b^+$ DC plus 3×10^3 $c\text{-}kit^+sca\text{-}1^+lineage^-$ HSC in combination with either 3×10^5 T-cells or no additional T-cells. In vivo donor-derived T-cell proliferation was assessed by CFSE staining. Serum and intracellular cytokines (Th1: IL-12, IFN-γ, IL-2, and TNF-α and Th2: IL-4, IL-5, and IL-10) were tested by ELISA and flow cytometry. Costimulatory molecule expression(CD40, CD80, ICOSL, PD-L1 and PD-L2) was measured by flow cytometry following recovery of GFP^+ donor DC on day_{+10} post-transplant. We found that donor CD8 T-cells had higher levels of Ki-67 expression and proliferation than donor CD4 T-cells 3 days post-transplant following transplantation with $CD11b^-$ DC compared with $CD11b^+$ DC, and without donor DC ($p < 0.001$). Both $CD11b^-$ DC and $CD11b^+$ DC had similar level of expression of CD40, CD80, and ICOSL in BM, in lymph nodes and in spleen at 10 days post-transplant, but $CD11b^+$ donor DC recovered from BM had much higher levels of PD-L1 than $CD11b^-$ DC, while $CD11b^-$ DC in all tissues had higher levels of PD-L2. Donor $CD11b^-$ DC enhanced Th1 cytokine production of donor T-cells, while donor $CD11b^+$ DC elevated Th2 cytokine production compared with T-cell alone. In conclusion: Donor $CD11b^-$ DC enhanced donor T-cell proliferation, especially CD8 T-cells compared with donor $CD11b^+$ DC or recipients of T-cells alone. Homing of donor DC to lymphoid organs and expression of costimulatory molecules on donor $CD11b^-$ and $CD11b^+$ DC subsets *in vivo* were similar, suggesting that the differences observed in donor T-cell activation and proliferation in distinct donor DC subset is likely due to local production of cytokines by donor DC. The limited GvHD seen with T-cells injected with $CD11b^-$ DC may be due to differential expression of PD-L2 compared with $CD11b^+$ DC.

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C-FLIP EXPRESSION DETERMINES LOW SENSITIVITY OF TYPE 17 T HELPER CELLS TO ACTIVATION-INDUCED CELL DEATH

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T cell apoptosis induced by repeated TCR stimulation, known as activation-induced cell death (AICD), plays an important role in T cell tolerance. Pro-inflammatory, IL-17-producing $CD4^+$ T cells (Th17 cells) have been recently identified as a unique T helper subset. Preliminary work from our lab indicated that Th17 cells participate in the pathogenesis of acute graft-versus-host disease (GVHD). In this study, we compared the susceptibility of polarized Th1 and Th17 effectors to AICD *in vitro* and *in vivo*. We found that Th17 effectors were significantly less susceptible to AICD upon TCR restimulation compared to Th1 effectors. It was further confirmed by Th17 effectors generated from *il17f/rfp* knock-in mice, RFP^+ (Th17) cells were indeed more resistant to AICD than RFP^- (non-Th17) cells. Resistance of Th17 cells to AICD was also observed when Th17 cells were co-cultured with Th1 cells *in vitro* or co-injected with Th1 cells *in vivo* in allogeneic recipients. To explore the molecular mechanism of AICD resistance in Th17 cells, we found that Th17 cells were defective in expression of FasL and in activation of caspases, but highly expressed anti-apoptotic protein c-FLIP as compared to Th1 cells. Given that Th17 cells were resistant to AICD when co-cultured with Th1 cells that express high levels of FasL, we hypothesized that Fas-signaling was impaired on Th17 effectors. After knocking down c-FLIP with its specific siRNA, Th17 cells upregulated FasL and underwent rapid apoptosis upon TCR restimulation, indicating that Th17 cells are resistant to AICD likely because the high level of c-FLIP prevents Fas-mediated apoptosis. These results suggest the resistance of Th17 cells to AICD as additional mechanism that contributes to the high pathogenicity of Th17 cells in autoimmune diseases and GVHD. Our finding also strengthens the rationale to use tumor-specific Th17 cells for adoptive cell therapy in cancer.

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MESENCHYMAL STEM CELL INFUSION AS PREVENTION FOR GRAFT REJECTION AND GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING FROM HLA-MISMATCHED DONORS: A PILOT STUDY

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Background: Allogeneic hematopoietic cell transplantation (HCT) following nonmyeloablative conditioning has been an effective treatment for many pts with hematological malignancies who have a HLA-matched related or unrelated donor. However, results of nonmyeloablative HCT in pts with HLA-mismatched donors have been disappointing due to high incidence of graft rejection and severe acute GVHD. Recent studies have suggested that infusion of mesynchymal stem cells (MSC) the day of HCT might promote engraftment and prevent acute GVHD after myeloablative allogeneic HCT. This prompted us to investigate whether MSC infusion a few hours before HCT could allow non-myeloablative HCT from HLA-mismatched donors to be performed safely (i.e. with a 100-day incidence of nonrelapse mortality <35%).